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A Systematical Study of Insecticide Residues on Vegetables

I. The Influences of Plant Varieties on the Deposition and Dissipation of Insecticides[1]

By

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Abstract : The after-spray deposition of insecticides on twenty-four different vegetables were compared and analyzed in regard to the morphological characteristics of plants at different growing stages. The total amount of insecticide deposited on plants is directly related to the surface-to-mass ratio (SMR) of that plant. The surface deposition rate of insecticide (SDR) has been calculated for all tested vegetables. Several exceptional high SDR vegetables due to their special plant configurations were detected in this study.

The rate of insecticide dissipation depends on the nature of insecticide and the influence of environmental factors as well as the plant structure. Tighter-structured vegetables tend to prevent the insecticide residues from the outside impacts and thus slow down the dissipation so as to leave more residues on marketable-size vegetables.

The dissipation patterns of same insecticide on six different small leafy vegetables were very similar and with only minor differences among them.

Of all the farming crops, the vegetable cultivation is particularly intensive in regard to labor, fertilizer and pesticides. Since the sub-tropical and tropical climates of Taiwan favors the insect population to flourish on vegetation, using insecticides to protect the crops from the insect attack is usually necessary. Pesticides not only increase the production cost but aslo create residual problem on marketed crops. To control the pesticide residues on vegetables under a tolerable limit in Taiwan is a hard work, because there are more than forty species or varieties of vegetables available in anytime of the year. More than that, most of the vegetables are grown in many small farms which were distributed in different regions. We are not sure that every farmer was aware of the

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undesirable consequence of too much residues were left on the vegetables

In order to acquire the needed information of pesticide residues on vegetable crops, the Pesticide Research Laboratory of Taiwan Agricultural Research Institute has decided to evaluate this important subject systematically. In this report, we presented the first part of investigation and that is the impacts of plant varieties and characteristics on the deposition and dissipation of pesticides.

Materials and methods

Tested crops and the experimental design : Total of twenty-four species or varieties were closely studied and the complete list was presented in Table 1. The age or the

Table 1. The chinese name, common name and scientific name of tested vegetables.

表1.供試蔬菜之中名，英文名及學名

Chinese name	Common name	Scientific name
莧菜	Edible amaranth	*Amaranthus mangestanus* L.
甜椒	Bell pepper	*Capsicum annuum* L.
扁蒲	Bottle gourd	*Lagenaria siceraria* (Mol.) Standl
青花菜	Broccoli	*Brassica oleracea* var. *botrytis* L.
花椰菜	Cauliflower	*Brassica oleracea* var. *botrytis* L.
芹菜	Celery	*Apium graveolens* L. var. *dulce*
結球白菜	Pe-tsai, chinese cabbage	*Brassica pekinensis* (Lour) Rupr.
芥藍	Chinese kale	*Brassica oleracea* L. var. *acephala* DC.
韮菜	Chinese leek	*Allium odorum* L.
甘藍	Cabbage	*Brassica oleracea* var. *capitata* L.
胡瓜	Cucumber (s. & l.)	*Cucumis sativus* L.
茄子	Egg plant	*Solanum melongena* L.
茼蒿	Garland chrysamthemum	*Chrysanthemum coronarium* L.
大蒜	Garlic	*Allium sativum* L.
球莖甘藍	Kohlrabi	*Brassica caulorapa* Pasq.
小白菜	Pai-tsai	*Brassica chinensis* L. var. *pai-tsai*
青梗白菜	Pak-choi	*Brattica chinensis* L. var. *pak-choi*
豌豆夾	Edible-podded pea	*Pisum sativum* L. var. *macrocarpon*
油菜	Field mustard	*Brassica campestris* L.
四季豆、菜豆	Snapbean	*Phaseolus vulgaris* L.
菠菜	Spinach	*Spinacia oleracea* L.
蕃茄	Tomato	*Lycopersicon esculentum* Mill.
蕹菜	Water convolvulus	*Ipomoea aquatica* Forsk
葱	Welsh onion	*Allium fistulosum* L.

maturity of the vegetables were determined by the weight of the plant rather than the length of growing period to avoid the seasonal fluctuation which may change the length of growing period. Samples were weighed and counted first and then the total plant surface was determined and recorded in a Li-cor area meter. ®

In the experiments of leafy and stem vegetables, the plants were seeded at four evenly spaced dates over their expected growing periods to provide the same vegetable of four different sizes for the insecticide treatment and the subsequent samplings. In this way, all environmental conditions of pre-sampling period would be the same for all treatments. The insecticide treatments were carried out whenever the largest plant reached the marketable size.

For those continually harvested vegetables such as cucumber, tomato, edible podded-pea, etc., all plots were planted at the same time, and the insecticide treatments were conducted when the production reached the peak stage. In this way, enough fruit-type vegetables of various sizes can be picked and sorted into four different sizes or age groups for the pesticide residue determinations and the dissipation study was not carried out in this group of vegetables.

Insecticide treatments and analysis : In order to minimize the possible bias, we tested two insecticides on all vegetables and counted on that two sets of data would provide a more reliable result. Carbaryl 85% w. p. and chlorpyrifos 25% w. p. were chosen and diluted to 1,200 and 1,000 fold respectively for spraying. The dilution for each compound was referred from the Plant Protection Manual of 1982 edition [1]. All insecticide sprays were performed in the morning before ten o'clock and the first sampling was collected three hours later, and other samples were collected on the 3rd, 6th, 9th, 12th and 15th day. One kilogram of vegetable was collected randomly from test plots and stored at −20°C before the chemical analysis. Samples were first macerated in a food chopper to obtain a homogenous blend, and from which three sub-samples of ten gram each were removed for extraction and clean-up. Analysis results were the average of three replicates.

Carbaryl analysis : each sub-sample was extracted by blending in 50 ml acetonitrile for one minute followed by filtration. The cake was extracted one more time in another 25 ml acetonitrile. Two filtrates were combined and concentrated to 30 ml under reduced pressure before transferred into a 500 ml flask and mixed with 200 ml 2% NaCl solution. The mixture was extracted with dichlormethane three times at volumes of 50 ml, 25 ml and 25 ml respectively. Three dichlormethane extracts were combined and evaporated to dryness before adding 10 ml n-hexane to dissolve the residue in the bottle for further clean-up. Next, the n-hexane solution was loaded in charcoal florsil column and washed with 50 ml of 10% ether in n-hexane mixture. The column was then eluted with 110 ml 20% n-hexane in dichlormathane. The first 40 ml aliquots was discarded and the next 60 ml was collected for the carbaryl residue determination. The solution was evaporated to dryness before proceeded to the trifluoroacetic anhydride (TFA) derivatization after the Lee's method [2] with certain modifications. The sample flask was first added 0.2 ml TFA, 0.1 ml pyridine and 0.2 ml ethyl acetate then mixed well to react in room

temperature for 30 minutes. After incubation, added 0.3 ml ether and 5 ml n-hexane into the sample and shaked well, then added 10 ml distilled water and shaked again. Once the separation of organic and aqueous phases were completed, the organic layer was pipetted into a dry glass vessel containing sodium sulfate anhydrous to remove water and the solution was ready for the GLC analysis.

Chlorpyrifos analysis : sub-samples were extracted by blending with 50ml methanol for one minute followed by filtration. The cake was extracted one more time in 25 ml methanol and the combined filtrate was ready for the clean-up. The filtrate and 200 ml 2% NaCl were mixed in a 500 ml separation funnel with 50 ml n-hexane. The mixture was shaked hard for one minute before separating the n-hexane fraction. The procedure was repeated two more times and each time with 25 ml fresh n-hexane and then combined three n-hexane fraction in a 500 ml flask, followed by adding 4 gram of absorbant mixture (charcoal : celite : sodium sulfate anhydrous at 1 : 1 : 14) and then shaked for 30 minutes on a shaker before filtering. After recorded the total volume of filtrated solution, the samples were submitted to the GLC determination.

Residues determination and recoveries : instrumental parameters for each compound are listed in Table 2. Recovery of chlorpyrifos was checked by fortification of untreated chinese kale, pak-choi and egg plant. The recovery of carbaryl was checked by fortification of untreated field mustard, cabbage, snap-bean and egg plant. The average recovery ranges were listed in Table 2. Residue data presented in this report were corrected for recovery loss.

Table 2. Instrumental parameters and recovery ranges
表2.測定儀器之操作情況及條件，以及回收率

Compound	Column temperature	Carrier gas pressure	Recovery
Carbaryl	200°C	8 lb/in²	93% (Field mustard)
			88% (Cabbage)
			87% (Snapbean)
			86% (Egg plant)
Chlorpyrifos	225°C	12 lb/in²	82% (Chinese kale)
			84% (Pak-choi)
			83% (Egg plant)

other parameters were same for both compounds :
column packing : 3% SE-30 on 100-120 mesh diatomite CQ 1.5m×4mm
injection port temperature : 250°C
detector : electron capture detector
detector temperature : 300°C.

Results

The morphological characteristics of different vegetables of various growing stages were carefully measured and analyzed. The surface-to-mass ratio (SMR) in cm^2/g depends on the plant size and the ratio usually decreases as the plant grows. We have estimated the relationship between the SMR and the plant size of fifteen different vegetables and their regressions were lised in Table 3. In order to make things more comparable among all vegetables, the data of cabbage is chosen to be the common standard in the following analyses and discussion. The SMR, the insecticide detained and the plant size of twenty-four tested vegetables were listed in Table 4. The depositions of both carbaryl and chlorpyrifos on plants have been presented in terms of surface deposition rate (SDR) in ug/cm^2 which represents the insecticide-holding capability of unit plant surface. According to their SMR values, vegetables could be classified into different groups. For examples, the SMR of cabbage is 7.0, if its outer-spread leaves were separately measured from the cabbage head in SMR, two values were 18.7 and 0.8 respectively. When they were compared to the other vegetables in Table 4, the first SMR resembled the small leafy vegetables and the second SMR stood perfectly within the values of fruit-type vegetables. If we examine the column of residues in Table 4, the fact is that the amount of residues followed closely with the magnitude of SMR. Therefore, the available plant surface is the most important factor in determining the amount of initial deposition.

Table 3. The regression of the SMR and the plant weight of tested vegetables

表3. 植株成長中之表面積與重量變化之相關方程。

Plant	Regressional equation	P
Pai-tsai	y=48.7−0.9086x	0.07360
Field mustard	y=44.3−0.6385x	0.04804
Celery	y=42.7−0.1834x	0.00008
Pak-choi	y=42.6−0.9448x	0.00004
Spinach	y=37.1−0.2996x	0.00797
Chinese leek	y=34.4−2.0076x	0.00017
Chinese kale	y=31.6−0.2312x	0.00000
Egg plant	y=18.1−0.0664x	0.17023
Garlic	y=17.8−0.2914x	0.00243
Snapbean	y=5.19−0.2637x	0.03321
Bell pepper	y=2.61−0.0107x	0.00983
Cucumber	y=1.81−0.0022x	0.01208
Bottle gourd	y=1.71−0.0009x	0.00272
Tomato	y=1.33−0.0037	0.00168

y=the SMR in cm^2/g. x=the plant weight in g.

no regressional equation can be established for the garland chrysamthemum and the edible-podded pea,

Table 4. The SMR, the total residue and the SDR of carbaryl and chlorpyrifos on tested vegetables.
表4. 供試24種蔬菜之收穫重量及所測定之表面積重量比，全部着藥量及單位面積着藥量

Plant	SMR	Carbaryl		Chlorpyrifos		Vegetable
	cm^2/g	ppm	SDR	ppm	SDR	Size, g
Cabbage	7.0	1.7	0.24	2.6	0.37	1,415.0
Cabbage (outer)	18.7	—	—	11.1	0.60	445.2
Cabbage (head)	0.8	—	—	0.1	0.14	969.8
Edible amaranth	53.9	120.0	2.23	84.2	1.56	5.8
Celery	38.2	49.5	1.30	9.3	0.26	19.4
Pai-tsai	36.0	12.5	0.35	17.4	0.48	13.1
Water convolvulus	35.0	45.9	1.31	16.8	0.48	11.6
Spinach	27.7	9.8	0.35	15.3	0.55	31.4
Field mustard	27.6	10.6	0.39	9.8	0.36	28.9
Pak-choi	27.3	14.9	0.54	15.1	0.55	9.5
Chinese kale	27.0	17.3	0.64	24.1	0.89	20.4
Garlic	26.2	13.5	0.51	5.2	0.20	21.3
Garland chrysamthemum	25.6	10.7	0.42	13.5	0.53	20.6
Edible-podded pea	9.2	2.5	0.27	3.2	0.35	1.9
Egg plant	8.9	0.9	0.10	0.4	0.04	139.2
Snapbean	2.2	2.1	0.97	0.8	0.37	13.9
Bell pepper	1.7	10.5	6.08	1.4	0.80	80.4
Cucumber, small	1.0	0.8	0.80	0.2	0.22	278.4
Cucumber, large	0.7	0.9	1.27	0.3	0.42	587.3
Bottle gourd	0.6	1.6	2.83	0.3	0.49	1,018.8
Tomato	0.6	1.2	2.00	0.15	0.24	215.8
Kohlrabi	0.5	trace	trace	0.6	1.24	613.8
Chinese leek	147.9	—	—	13.2	0.09	9.5
Welsh onion	75.6	—	—	3.8	0.05	17.3
Cauliflower	—	7.1	—	2.8	—	442.3
Broccoli	—	6.2	—	3.2	—	370.0

The aspect that we did not explored in this study is the individual effect of fine structure and configuration of plants. But several indications in this aspect can be found in Table 4. For example, the egg plant is extremely low in SDR which matched the fact that insecticide spray can not stay on the silky-smooth surface. On the other hand, although the bell pepper also has the waxy surface, the concave groove a configuration factor-around the sepal of marketable size bell pepper can withhold three to six milli-litres of insecticide sprays and hence raises its SDR reading.

During the chemical analysis, both welsh onion and chinese leek produced certain unknown chemicals which interfered the analysis of carbaryl and hence could not provide two sets of residue information as we wanted for investigation. Nevertheless, attention should be paid to welsh onion and chinese leek due to their high SMR which implicated the potential hazard of high residue if the insecticides were used on them. In order to obtain a more objective view, we compared the SMR, SDR and the total deposition of insecticide of all tested vegetables to those of cabbage to obtain relative values of SMR, SDR and total deposition. The final figures of r-SDR and r-residue were the average of both carbaryl and chlorpyrifos tests and were listed in Table 5.

Table 5. The relative-residue, the relative-SMR and the relative-SDR of twenty-four vegetables compared to cabbage.

表5. 供試 24 種蔬菜測定數據對甘藍菜之相對值

Plant	r-Residue	r-SMR	r-SDR
Cabbage	1.00	1.00	1.00
Cabbage (outer)	—	2.67	—
Cabbage (head)	—	0.11	—
Edible amaranth	51.49	7.71	6.68
Water convolvulus	16.73	5.02	3.33
Celery	16.45	5.46	3.01
Chinese kale	9.73	3.87	2.51
Pak-choi	7.28	3.92	1.86
Pai-tsai	7.02	5.15	1.36
Spinach	5.82	3.97	1.47
Garland chrysamthemum	5.74	3.67	1.56
Field mustard	5.01	3.96	1.27
Garlic	4.96	3.75	1.34
Bell pepper	3.37	0.24	13.74
Edible-podded pea	1.35	1.31	1.03
Snapbean	0.78	0.31	2.52
Bottle gourd	0.51	0.08	6.56
Tomato	0.37	0.09	4.26
Egg plant	0.34	1.28	0.27
Cucumber, large	0.33	0.10	3.30
Cucumber, small	0.27	0.15	1.86
Kohlrabi	0.12	0.07	1.71
Chinese leek	—	21.13	—
Welsh onion	—	10.80	—
Cauliflower	2.62	—	—
Broccoli	2.43	—	—

We traced the r-SDR of several vegetables at different growing stages and the general tendency indicated that younger plants were high in r-SDR compared to old or more matured plants. It seems that the tighter configuration and hairier surface of young plant resulted in this phenomenon and the test results were presented in Fig. 1.

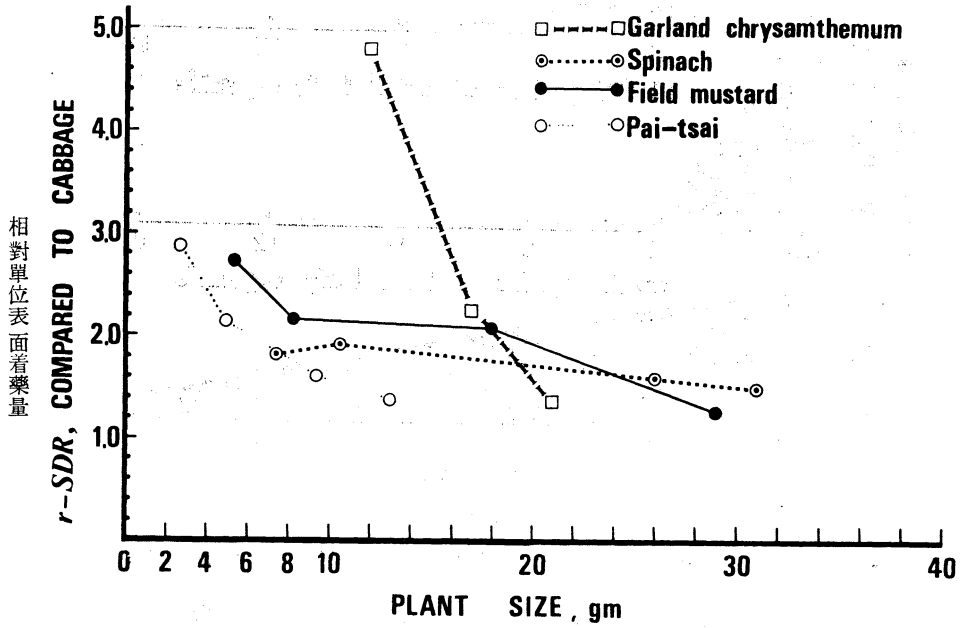

Fig. 1. The variation of r-SDR of four small leafy vegetables in relation to plant size.

圖1. 小葉菜之相對單位表面着藥量隨生長變化之情形

In the insecticide dissipation study, six different small leafy vegetables were treated with carbaryl and chlorpyrifos separately and the dissipation patterns of both insecticides were compared in Fig. 2 (a) and 2 (b). Although the tests were carried out in different seasons which covered a period of six months, the dissipation of chlorpyrifos in all six small leafy vegetables were very close. After six days, the chlorpyrifos residues fell to only 5% of what that plant initially received. The disappearance of carbaryl in same six vegetables shown greater variation than that of chlorpyrifos in the first week, but at the end of the ninth day, the carbaryl residues in all six vegetables fell below 10% of what plant originally received. In general, the dissipation of carbaryl is slower than that of chlorpyrifos. Since both insecticides were tested in the same time and in the same field, the difference may due to their formulations or physical-chemical natures.

However, the dissipation of both insecticides on small leafy vegetables were far apart from the disappearing pattern of chlorpyrifos on large leafy vegetables. The dissipation of chlorpyrifos on cabbage and chinese cabbage were so similar and were combined in Fig. 2 (c). The slow disappearing rates of insecticide on both tight-structured vegetables reaffirmed the need of more study in plant-specific factors when we investigate the

residue behavior on different vegetables.

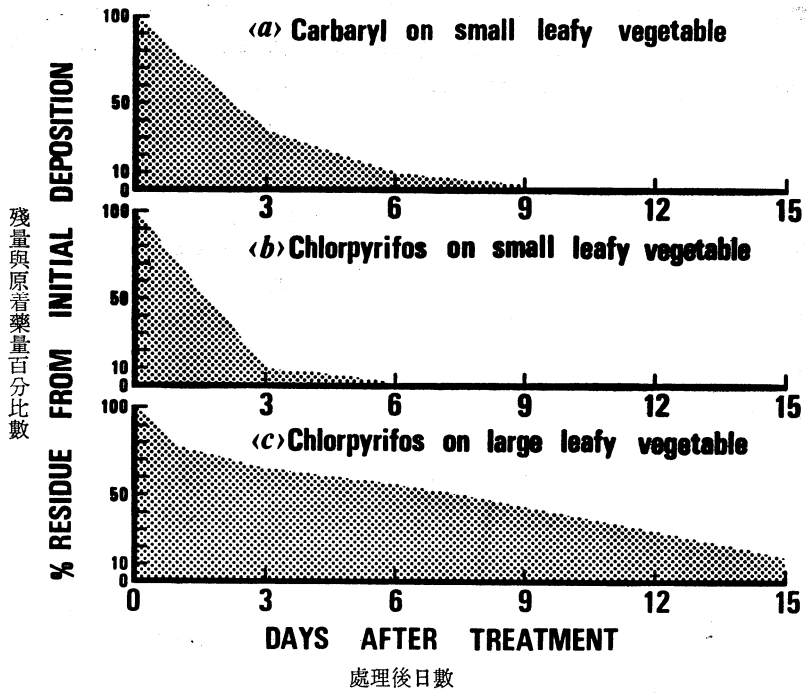
(a) Carbaryl on small leafy vegetable
(b) Chlorpyrifos on small leafy vegetable
(c) Chlorpyrifos on large leafy vegetable
% RESIDUE FROM INITIAL DEPOSITION
殘量與原着藥量百分比數
DAYS AFTER TREATMENT
處理後日數

Fig. 2. The comparison of dissipations of two insecticides on small and large leafy vegetables.
(a) averaged results of carbaryl dissipation on six small leafy vegetables.*
(b) averaged results of chlorpyrifos dissipation on six small leafy vegetables.
(c) averaged results of chlorpyrifos dissipation on cabbage and chinese cabbage.
* six small leafy vegetables were spinach, chinese kale, pai-tsai, field mustard, garland chrysamthemum and pak-choi. testing period covered a period from September to March.

圖2. 殺蟲劑在小葉菜及包葉菜上消退情形之比較

Discussion

In this investigation, we intended to find out the information of insecticide retaining ability, the biological dilution factor and the residue dissipation patterns of different vegetables. In hoping to establish a predicting system to estimate the possible fate of an insecticide of known characteristics on a particular vegetable crop. Such a system will save time and labor by merely testing several selected vegetables under the standard experimental condition. The test results will provide information for an accurate residue prediction on other vegetables. Since the system is designated for the practical use, no attempts have been made to investigate any particular plant morphology which may influence the deposition and persistence of insecticides.

Results of this study have clearly indicated that the variation of residue on a plant is more complicated than expected. The fact can be verified by simply examining the wide range of measurements which we obtained in both plant characteristics and residues. Another difficulty about the pesticide residue investigation is that the amount of residue

which deposited on a target plant and its dissipation are dynamic matters and they relied upon both the changing morphology of growing plants and the variation of environmental conditions. Bewaring the residue problem might have different degree of seriousness on various vegetables, Braun et al.[3] examined the dissipation of commonly used insecticides in six minor vegetable crops in Canada and made certain comparison among plant morphology in relation to the dissipation of residues. Unfortunately, not many other works in this regard have been done so far.

In general, we believed that the attention should be paid to the SMR and the SDR of a target vegetable. The cucumber and the egg plant have provide distinctive examples in illustrating our opinion. The cucumber is high in SDR and low in SMR, on the other hand, the egg plant is low in SDR and high in SMR and the combined results indicated that the egg plant has retained similar amount of residue as that of cucumber, even though the two main contributing factors were stood at opposited extremes.

We would also like to point out that the plant characteristics are not the only important factor in determining the possible hazard of residues. For example, amaranthus and water convolvulus have high SMR readings and SDR values, but both vegetables were scarcely in need of insecticide treatments in cultivation. On the other hand, cruciferous group vegetables were constantly in need of insecticide treatments to relief threats of many serious insect pests in Taiwan, and they possessed greater threat in residue hazards.

Currently, the cabbage is the most tested vegetable in providing the pesticide residue information for the registration purpose. From what has been found in our investigation, it is clear that cabbage is not particiulaly good in representing neither the total deposition nor the dissipation of a tested insecticide on many other vegetables. We therefore suggested that at least one model vegetable should be selected from each morphologically related group for the insecticide residue investigation and this viewpoint is also shared by the recommendation of Codex Committee on Pesticide Residues[4]

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蔬菜農藥殘留問題之研究

I. 蔬菜性狀對農藥粘着及消退之影響[1]

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摘 要

臺灣省農業試驗所農藥研究室針對蔬菜易於發生農藥殘留之問題，進行此一系統研究與分析，此文爲第一部分研究結果，乃就24種蔬菜之性狀及施藥後着藥量多少，消退快慢進行分析。研究對象之24種蔬菜包含小葉菜，大葉菜（含包葉菜），豆菜及茄，甜椒，瓜等，測定項目包含與各種蔬菜着藥量有重要關係之表面積與質量比（SMR），全部着藥量（total residue），單位表面着藥量（SDR），成株重量等，全部資料可用於將來成立系統分析所需。試驗時又設定甘藍菜之各項數據爲標準，而換算其他各種蔬菜之各項數值，成爲相對表面積與質量比（r-SMR），相對着藥量（r-residue）及相對單位表面着藥量（r-SDR），易於判定各項因子之大小與特質（以上資料均詳列於表四與表五之中）。試驗結果證實 SMR 值與着藥量成正比，再由 SDR 偏高之蔬菜中，可看出其性狀及表面構造有某些特點有利於聚集藥液，如茄子表面光滑，而其 SDR 值亦低，甜椒表面光滑，但其 SDR 值最高，原因在於甜椒果柄部分之周圍有一環狀之凹陷，在施藥後可聚集 3～6 c.c. 的藥液，而大幅提高其 SDR 值。詳細分析全部資料可檢出降低或提高農藥殘留問題之植物性狀因子，供以後育種參考之用。隨植株之成長， SDR 值亦因而降低，原因可能係植物之幼莖幼葉多毛並常卷曲，而易於聚凝藥液，從而導致單位表面積之粘着量昇高。

就殘量之消退而言，其速率隨不同殺蟲劑之配方，主成分之理化性狀而有差異。本試驗中陶氏松（chlorpyrifos）之殘量消退速率即較加保利（carbaryl）爲快，而包葉菜的緊密構造易於保護殘留於內部之藥液，因而降低其分解速率，由此可見在蔬菜殘留農藥問題上，植物本身性狀是一項重要的決定因子，可大幅影響其對農藥之粘着及消退之速率。

1.臺灣省農業試驗所 研究報告第 1112 號。本研究爲中央加速農建補助計畫〔70農建—5.1—產—01(02) 與71農建—4.1—產—39 (05)〕承農業發展委員會補助研究經費，簡淑貝及簡珠碧協助研究工作，謹此致謝。

2.本所應用動物系助理及研究員兼系主任。臺灣省 臺中縣 霧峰鄉。